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PRINCIPAL INVESTIGATOR: Ercole L. Cavalieri, Ph.D.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-7835

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6. AUTHOR(S)
Ercole L. Cavalieri, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Nebraska Medical Center
Omaha, Nebraska 68198-7835

E-Mail: ecavalie@unmc.edu

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13. ABSTRACT (Maximum 200 Words)

Treatment of Noble rats with testosterone plus estradiol (E_2) induces prostate carcinomas. We think that estrogens initiate prostate cancer by reaction of catechol estrogen-3,4-quinone (CE-3,4-Q) metabolites with DNA. Formation of depurinating adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate prostate cancer. After treatment of rats with CE or CE-3,4-Q, CE metabolites and CE-glutathione (GSH) conjugates were lower in regions where tumors develop and methoxyCE were higher in regions where tumors do not develop. To study the role of CE-Q in initiation of prostate cancer, we are (1) treating rats with E_2 and currently analyzing the CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts in the regions of the prostate by HPLC with electrochemical and mass spectrometric detection; (2) studying in the prostate conversion of testosterone into E_2 and its metabolism; and (3) currently determining the expression at the mRNA level of four selected enzymes involved in estrogen activation and deactivation in the prostate of rats treated with testosterone. These studies will provide information critical to understanding the molecular etiology of prostate cancer, identify biomarkers for early detection of susceptibility and lead to development of strategies for prostate cancer prevention.

14. SUBJECT TERMS
estrogens as endogenous tumor initiators; initiation of prostate cancer in Noble rats by estrogens; analysis of estrogen metabolites, conjugates and DNA adducts; expression of estrogen activating and deactivating enzymes

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Introduction

The purpose of this research is to investigate the hypothesis that estradiol (E_2) initiates prostate carcinogenesis and testosterone promotes the process. This is being explored in male Noble rats, which develop prostate tumors when treated with E_2 and testosterone [1]. We think that estrogens are involved in the initiation of prostate cancer by a mechanism that involves oxidation of endogenous 4-catechol estrogen (CE) metabolites to CE-3,4-quinones (CE-3,4-Q). Reaction of CE-3,4-Q with DNA results in tumor initiation as the first step in the events leading to prostate cancer. Formation of depurinating DNA adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading mutations that initiate the cancer [2]. To study the role of CE-Q in the initiation of prostate cancer, we are (1) treating male Noble rats with E by i.p. injection at various doses and for various times, analyzing the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts and comparing their levels in the various regions of the prostate [3]; (2) investigating the conversion of testosterone into E_2 in the prostate by analyzing the same compounds in prostate tissues from rats treated with testosterone or testosterone plus the aromatase inhibitor letrozole; and (3) determining the expression of four enzymes involved in the activation and deactivation of estrogens, cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase (COMT) and quinone oxidoreductase (QOR). The results of these studies will provide information on the relationship between estrogen activation and deactivation in relation to tumor initiation in the prostate.

Body

In the first year of this research project, significant progress has been made on the projected tasks, as detailed in the Statement of Work. Because the analyses are in progress as this report is being written, we do not yet have results to report. We can report, however, that data are being successfully acquired.

Task 1: Conduct the E_2 dose-response study of CE metabolites, GSH conjugates and DNA adducts.

The animals were treated with 0, 16, 32 or 48 mg/kg of E_2 by i.p. injection, and after 3 h the prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection are currently being conducted.

In addition, animals were treated with testosterone by implantation for 2 wk or by i.p. injection of 0 or 52 mg/kg for 6 h. The prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection are currently being conducted. The dose-response and time course experiments with testosterone will be conducted based on the results of this study.

Task 2: Conduct the E_2 time course study of CE metabolites, GSH conjugates and DNA adducts.

Initiation of this task awaits the results of the E_2 dose-response study, and it will be conducted in the next few months.

Task 3: Synthesize primers for analyses of mRNAs.

This has been accomplished and the primers are being used to analyze expression of the enzymes.

Task 4: Analyze the expression of estrogen-metabolizing enzymes in control animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in control rats is currently being conducted.

Task 5: Begin analysis of the expression of estrogen-metabolizing enzymes in E_2-treated animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in rats treated with testosterone (as described in Task 1) is currently being conducted. This will be followed by analysis of the enzymes in E_2-treated animals.

Key Research Accomplishments

1. Groups of rats were treated with estradiol (3 different doses injected for 3 h) or testosterone (implanted for 2 wk or injected for 6 h), the prostates were excised and dissected into the dorsolateral prostate, ventral prostate, seminal vesicle, coagulating gland and urethra, and the tissues were shipped to UNMC for analysis.

2. Tissues from the testosterone experiment are being analyzed for expression of the estrogen-metabolizing enzymes cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase and quinone oxidoreductase at the mRNA level.

3. Tissues from the testosterone experiment are being analyzed for the levels of estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts by HPLC with electrochemical and mass spectrometric detection.

Reportable Research Accomplishments

None thus far.

Conclusions

In this first year, we have established the minimum amounts of tissue needed to extract RNA successfully and to analyze estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts. We have also established the protocol necessary to extract tissues for analysis of estrogen derivatives by HPLC. In addition, we have found a compound to serve as an internal standard, so that we can validate the level of recovery of estrogen derivatives from tissue samples. These parameters are all necessary so that the results of these studies are as precise and meaningful as possible.

References

1. Bosland, M.C., Ford, H., and Horton, L. Induction at high incidence of ductal prostate adenocarcinomas in NBL/Cr and Sprague-Dawley Hsd:SD rats treated with a combination of testosterone and estradiol-17β or diethylstilbestrol. Carcinogenesis, 16: 1311-1317, 1995.

2. Cavalieri, E.L., Rogan, E.G. and Chakravarti, D. Initiation of cancer and other diseases by catechol ortho-quinones: A unifying mechanism. Cell & Mol. Life Sci., 59: 665-681 2002.

3. Cavalieri, E.L., Devanesan, P., Bosland, M.C., Badawi, A.F. and Rogan, E.G. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: Implications for estrogen-induced initiation of prostate cancer. Carcinogenesis, 23: 329-333, 2002.